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Our preclinical studies in tumor-bearing mice have shown that intratumoral injection of an adenoviral vector expressing interleukin-12 was effective in inducing tumor regression, antitumor immune responses and long term survival without disease relapse in 40% treated animals. Based on these findings, we have developed a Phase I trial of intratumoral delivery of an adenoviral vector (Adv.RSV-hIL12, ADV-hIL12) expressing the human interleukin-12 cDNA in patients with metastatic breast cancer to the liver. We have completed preclinical efficacy and toxicity studies to support an IND application to the FDA to conduct the proposed clinical trial. Revisions to the clinical protocol and consent have been made on the recommendations of the FDA and NIH-OBA. We have successfully completed clinical grade production of the study agent (Adv.RSV-hIL12) in the Vector GMP Facility at Mount Sinai. The IND was approved by the FDA (6/11/02). The revised protocol and consent was submitted to the Department of the Army (6/17/02) for approval. The Human Subjects Protection Division of the Department has granted approval on 10/17/04 after further revisions were made at their request. The clinical trial will be activated for patient accrual once the revisions requested by the HSPD have been approved by the local IRB.

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INTRODUCTION

Interleukin-12, a heterodimeric cytokine secreted by activated monocytes and macrophages, has been shown in murine tumor models to inhibit tumor growth, induce gamma interferon (IFNγ) production, and modulate the immune reactivity of natural killer and cytolytic T-cells. We have constructed an adenoviral vector which expresses murine interleukin-12 cDNA under the control of the Rous sarcoma virus promoter (Adv.RSV-mIL12) (1, 2). We have shown in mice bearing established metastatic tumors (breast JC and colon MCA26) in the liver that intratumoral injection of these tumors with the IL12-expressing vector resulted not only in tumor regression, but also in long term survival in up to 40% of treated animals (1-3). We have also completed preclinical toxicity studies and have shown the vector to be well tolerated at therapeutically effective doses (5). We have translated our preclinical results into a Phase I clinical trial of intratumoral injection of an adenoviral vector which expresses the human interleukin-as cDNA (Adv.RSV-hIL12 or ADV-hIL12) in patients with metastatic breast cancer to the liver. We have constructed the adenoviral vector expressing human interleukin-12 (ADV-hIL12) for clinical grade production of the study agent to be used in the proposed clinical trial (4). The vector will be injected by percutaneous skinny needle placement in a liver metastasis under ultrasound guidance and local anesthesia. The dose of ADV-hIL12 will be escalated in cohorts of three patients each. Patients will be treated and monitored in the General Clinical Research Center for assessment of toxicity. Followup monitoring will include assessment of delayed toxicities, immune and tumor responses.

BODY

Report on Work Performed During Period 10/1/03-9/30/04

The major goal in this project is to conduct a Phase I trial of ADV-hIL12 administered by intratumoral injection in patients with metastatic breast adenocarcinoma to the liver. The tasks which needed to be completed are summarized below.

- ADV-hIL12 production, certification and approvals
  - Arrange for production of ADV-hIL12 under GMP conditions.
  - Obtain certification testing of ADV-hIL12 for clinical applications by FDA-approved vendors.
  - File IND application with the FDA and the Office of Biotechnology Activities of the National Institutes of Health (NIH-OBA).
  - Obtain approvals from all local and federal regulatory agencies.

- Conduct Phase I trial of ADV-hIL12 in patients with hepatic metastases from breast cancer
  - Implement and complete Phase I trial.
  - Analyse data from Phase I trial.

ADV-hIL12 Production, Certification and Approvals

As described in the previous annual reports, we have completed production of a clinical grade preparation of ADV-hIL12 in November 2002. Samples from the production lot were sent to Charles River Associates and Galbraith Laboratories, independent FDA-approved testing agencies for lot release testing. Complete sequencing of the vector was also performed at the Mount Sinai School of Medicine. The testing was completed and results forwaded to the FDA on April 12, 2002. The production lot was approved by the FDA for use in the proposed clinical trial in a letter dated June 11, 2002.

Regulatory Approvals

With approvals obtained from the FDA, NIH-OBA, Mount Sinai IRB and IBC, we submitted the final version of the protocol and consent to the Department of the Army and its Human Subjects Protection Division (HSPD) for their approval on June 17, 2002. Following revisions made at the request of HSPD/USAMRMC, the protocol and consent has been approved by HSPD on 10/17/04. The revisions have been submitted to the Mount Sinai School of Medicine IRB for approval. The clinical trial will be activated once approval from the IRB has been obtained.

Extension of Grant

Because of unanticipated delays in approval of the amended protocol and consent from the Department of the Army, we have requested from the USAMRAA a no-cost extension to the grant for the period 10/1/2004 to 10/1/2005.

KEY RESEARCH ACCOMPLISHMENTS

- Demonstration of tumor regression and survival prolongation following intratumoral injection of ADV-mIL12 in a murine orthotopic model of established breast cancer metastasis in the liver.

- Demonstration of safety at therapeutically effective doses of ADV-mIL12 administered by intratumoral injection in tumor-bearing mice.

- Demonstration of high levels of IL12 in tumor following intratumoral ADV-mIL12 injection in tumor-bearing mice, but low levels of IL12, IFNγ, and proinflammatory cytokines IL6 and TNFα in the serum.

- Demonstration of absence of detectable systemic dissemination of ADV-mIL12 following intratumoral injection in tumor-bearing mice.

- Approval of the clinical protocol and consent by the FDA, NIH-OBA, and NIH-RAC.

- Determination that the plasmid pJM17 will have to be used instead of pBHG10 to generate the recombinant vector to reduce the level of replication competent adenovirus.

- Demonstration that the toxicity of high doses of the adenoviral vector expressing IL12 in tumor-bearing animals was due to the transgene product rather than to the adenovirus particles.

- Production of the clinical grade vector ADV-hIL12 for use in the proposed trial.

- Approval from the FDA for the clinical grade ADV-hIL12 production lot for use in the proposed trial and to proceed with activation of the trial for patient accrual.

REPORTABLE OUTCOMES

- Preclinical efficacy and toxicity studies of adenoviral vector expressing interleukin-12 when administered by intratumoral injection in mice bearing established metastatic cancer in the liver.

- Manufacture of ADV-hIL12 seed vector and production lot under GMP conditions.

CONCLUSIONS

The work which has been performed on this project to date indicate that translation of laboratory studies to the benchside can be accomplished. Our preclinical studies in an orthotopic murine model of established breast cancer metastasis in the liver have shown that intratumoral injection of an adenoviral vector expressing interleukin-12 was effective in inducing tumor regression and long term survival without disease relapse in 40% of treated animals. We have since performed additional preclinical efficacy studies and a complete series of preclinical toxicity studies to support the clinical translation to a clinical Phase I trial. We have constructed and produced clinical grade preparations under GMP conditions the adenoviral vector study agent (ADV-hIL12) to be used in the clinical trial. The clinical grade preparation was extensively tested by independent outside testing agencies and passed lot criteria mandated by the FDA. We have obtained IND approval from the FDA to conduct the proposed Phase I clinical trial using the clinical grade preparation of ADV-hIL12 as described in the clinical protocol and consent, which also included revisions requested by the FDA. The protocol and consent has been recently approved by the Human Subjects Protection Division of the USAMRMC (10/17/04) and will be activated once IRB approval has been obtained.

The completion of the clinical trial will provide us with important information on the feasibility and safety of adenoviral mediated delivery of interleukin-12 to tumor tissue and on its efficacy in inducing tumor regression and immune responses. The results of the trial will provide the basis for a subsequent clinical trial of ADV-hIL12 in combination with systemic administration of the recombinant human fusion ligand of the human 4-1BB T-activator protein (hIg-h4-1BB-Ls), which has been shown in preclinical studies to significantly enhance the efficacy of ADV-hIL12.

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